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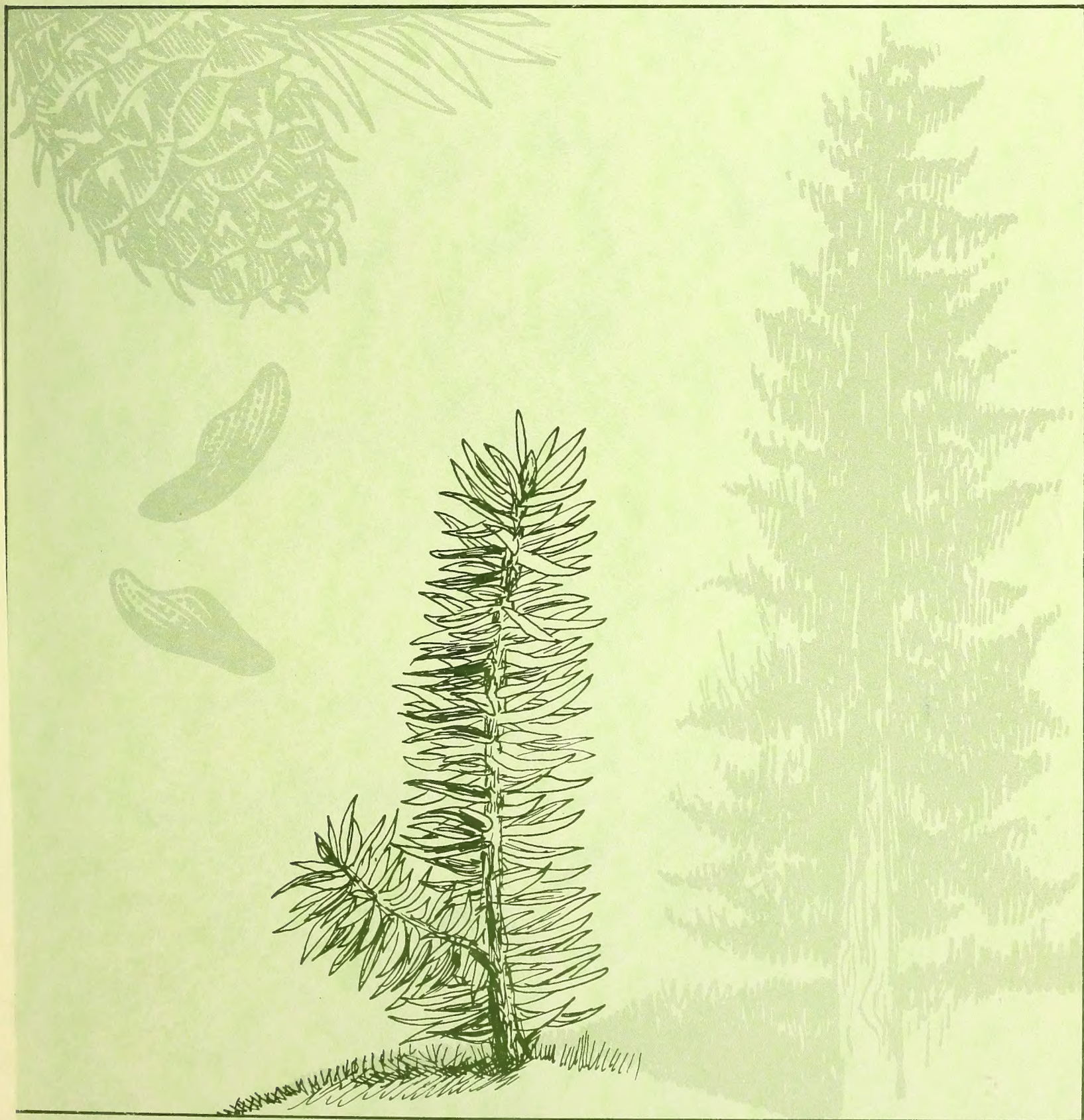
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Effects of Manganese and Manganese-Nitrogen Applications on Growth and Nutrition of Douglas-fir Seedlings

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English Equivalents

1 liter = 0.2642 gallon
1 kilogram = 2.2046 pound
1 gram = 0.0353 ounce
1 centimeter = 0.3937 inch
1 kilogram per hectare = 1.1206 pound per acre

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EFFECTS OF MANGANESE AND MANGANESE-NITROGEN APPLICATIONS ON GROWTH AND NUTRITION OF DOUGLAS-FIR SEEDLINGS

Reference Abstract

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Effects of manganese (Mn) on Douglas-fir grown in soil, with and without urea, and in nutrient solution were investigated. In addition, Mn sorption by forest soils was evaluated. Results show that Douglas-fir does not respond to added Mn and is quite tolerant to high Mn levels. Moreover, Mn sorption by soils is high. It is doubtful that Mn toxicity is of practical importance to production of Douglas-fir under forest conditions.

KEYWORDS: Soil manganese, nitrogen fertilizer response, seedling growth, Douglas-fir, Pseudotsuga menziesii. ✓

RESEARCH SUMMARY

Research Paper PNW-265

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Effects of different amounts of Mn as manganous sulfate on Douglas-fir grown in soil with and without urea fertilizer were determined. Effects of Mn were also studied when Douglas-fir was grown in nutrient solution. In addition, Mn sorption by different forest soil types was evaluated. Results show that Mn applied to the soil had no positive or negative effects on growth of seedlings regardless of urea additions. The highest amount of Mn tolerated without any visible sign of injury was 12 070 kilograms per hectare added to the soil and nearly 4,000 parts per million (p/m) in the foliage. Mn sorption by different soils was high; it varied among the soils studied and ranged from 30.8 to 64.0 percent. Mn toxicity in Douglas-fir grown in nutrient solution did not develop until Mn concentration in the solution reached 400 p/m. The seedlings tolerated 8,100 p/m in their shoots and 2,410 p/m in their roots. Because of high sorptions of Mn by different forest soils and high tolerance by Douglas-fir to accumulations of Mn in its shoots and roots, it is doubtful that Mn toxicity can be of importance under forest conditions, with or without urea fertilization.

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INTRODUCTION

Manganese (Mn) is an essential micronutrient for all plants, including Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco). Much is known about the importance of Mn in plant life and its functions in plants. Briefly, Mn is believed to be involved, at least indirectly, in the synthesis of chlorophyll (Meyer and Anderson 1952), a participant in the light reactions of photosyntheses (Heath and Hind 1969), an activator of many enzyme systems (Epstein 1972), and a factor in nitrogen metabolism (Jones et al. 1949).

Deficiencies and toxicities of Mn are well documented in the agronomic literature (e.g., Labanauskas 1966, Foy et al. 1978). Although Mn deficiency has been observed in shade and orchard trees on calcareous soils, reports of deficiencies in trees in forest situations are rare (Stone 1968). Conversely, the possibility of Mn toxicity in forest trees has been suggested in numerous instances. High levels of Mn in soils and tree tissues have been linked to reduced growth (Adams and Walker 1975), injury (Ross 1961), and death (Child and Smith 1960) of some forest tree species.

Concentrations of Mn have been determined in foliage of Douglas-fir trees and seedlings (Beaton et al. 1965, Krueger 1967). Deficiency levels of Mn, interaction of Mn with other nutrients--e.g., nitrogen (N)--and effects of Mn amendments on growth and nutrition of Douglas-fir, however, have not been determined. A low-level, chronic Mn toxicity might be postulated in Douglas-fir and other northwestern species for several reasons. Forest soils of the Douglas-fir region are quite acid and are saturated for much of the year; both of these factors enhance availability of soil Mn (Buckman and Brady 1969). Moreover, recently collected data on nutrition indicate a significant negative relationship between growth and amount of Mn in foliage of western hemlock (Tsuga heterophylla (Raf.) Sarg.) (Radwan and DeBell 1978) and Douglas-fir.^{1/}

In this paper, we report results from several experiments aimed at clarifying the status of Mn nutrition in Douglas-fir. We investigated effects of different rates of Mn applied to soil, with and without urea fertilizer, on growth of Douglas-fir seedlings, soil pH, and chemical characteristics of foliage of treated trees. We also studied availability of Mn in the soil and its sorption by different soil types, and the tolerance to and uptake of Mn by Douglas-fir seedlings grown in nutrient solution.

^{1/}Unpublished data on file at Forestry Sciences Laboratory, Olympia, Washington.

METHODS AND RESULTS

Experiment 1

We applied Mn and N at different rates to Douglas-fir seedlings and measured the effects of treatments on growth, soil pH, available Mn in soil, and chemical characteristics of the foliage.

Methods

Test seedlings.--Uniform, 3-year-old Douglas-fir seedlings from a low elevation Oregon seed source were used. The seedlings were started in the greenhouse in styrofoam planters in the spring of 1974 and moved outdoors in 1975. The seedlings were individually potted in 3.8-liter plastic pots in the winter of 1976. Each pot contained about 2.8 kg of Tumwater sandy loam soil. The native level of available Mn in this soil is quite low (17 p/m) compared with other soils used in our studies (42 to 136 p/m). Seedlings were watered with half-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) in April and then fertilized twice in the 1976 growing season with a urea solution at a rate equivalent to 56 kg N/ha. In April 1977, heights and diameters of seedlings were measured to determine size of individual seedlings prior to treatment. Seedlings were then numbered and assigned different treatments at random. There were 10 seedlings per treatment and a total of 80 plants.

Fertilization treatments.--Eight treatments (four levels of Mn and two levels of N) were tested. Manganese and nitrogen were applied as manganous sulfate and urea, respectively, and all chemicals were applied to the soil in solution. Application rates of N were equivalent to 0 and 168 kg/ha, and those of Mn were equivalent to 0, 28, 112, and 560 kg/ha. In May 1977, just before bud burst, seedlings were moved to a lathhouse and treatments were applied. In each case, the required amount of urea (contained in 50 ml solution) was added, followed by the specified amount of manganous sulfate (contained in 100 ml solution) and 50 ml of water. Throughout the growing season, seedlings were watered as needed without leaching; the plants were harvested in January 1978.

Soil pH measurements.--One pot of each treatment was used to periodically determine pH after treatment. Each time, soil collected with a sampling tube (to the full depth of the pot) was thoroughly mixed with distilled water (1:1) and pH of the suspension was measured with a glass electrode about 30 minutes later. Soils were returned to their respective pots after each measurement.

Growth measurements.--Height and diameter of seedlings in pots not used in the pH determinations (nine seedlings/treatment) were measured at the end of the growing season. At harvest, dry weights of shoots and washed roots were determined after they were dried at 65°C to constant weight in a forced-air oven.

Determination of available Mn in the soil after harvest.--For each treatment, three composite samples were obtained; each consisted of thoroughly mixed soil from three pots. Fifty-gram portions of each sample were extracted for 2 hours with DTPA (diethylenetriaminepentaacetic acid) (Lindsay and Norvell 1978). Mn in the filtered solutions was determined by atomic absorption (Perkin-Elmer Corporation 1976).

Chemical analyses of foliage.--Analysis was limited to current year's needles to determine total N, calcium (Ca), Mn, iron (Fe), and zinc (Zn). Needles were obtained from all parts of each seedling before the shoots were oven-dried. After drying, weights of needles to be used in the analyses were added to weights of the corresponding seedling shoots to obtain dry weights of shoots. Three composite samples of each treatment were analyzed; each sample consisted of needles from three of the nine seedlings receiving the treatment. Composite samples were individually ground to 40 mesh in a Wiley mill and stored in sealed containers at -15°C until needed for analysis. Total N (including nitrate) was determined by the micro-Kjeldahl procedure (Bremner 1965); Ca, Mn, Fe, and Zn by atomic absorption (Perkin-Elmer Corporation 1976).

Statistical analysis.--Except for pH values, all data were subjected to analysis of variance; and means were separated by Duncan's multiple range test.

Results

Growth characteristics.--Data on growth of the treated seedlings are shown in table 1. As expected, all growth characteristics of seedlings were enhanced by nitrogen fertilizer, and needles of seedlings receiving added N were greener than those not fertilized with urea. Manganese, on the other hand, had no effect on height growth or shoot weight. Diameter growth and root weights were similarly unaffected in the absence of N fertilizer. However, diameter growth of the fertilized trees receiving 112 kg Mn/ha were significantly lower than those receiving 560 kg Mn/ha. Also, root weights of N-treated seedlings receiving 28 and 112 kg Mn/ha were significantly lower than those of seedlings not treated with Mn. These differences are unlikely to be real, because diameter growth and root weights of nitrogen-fertilized seedlings treated with 560 kg Mn/ha were not significantly different from those with no added Mn. Furthermore, visible symptoms of injury were not observed in any treatment.

Table 1--Growth characteristics of Douglas-fir seedlings treated with manganese and nitrogen^{1/}

Treatment		Height growth	Diameter growth	Shoot weight	Root weight
Mn	N				
Kilograms per hectare		- - - Centimeters - - -	- - - - - Grams - - - -		
0	0	14.6 a	1.73 a	18.3 a	11.2 abc
28	0	16.0 a	2.05 a	19.8 a	11.2 abc
112	0	15.8 a	2.00 a	20.5 a	10.5 ab
560	0	14.7 a	2.03 a	19.2 a	10.2 a
Average		<u>15.3 x</u>	<u>2.00 x</u>	<u>19.4 x</u>	<u>10.8 x</u>
0	168	22.1 b	3.25 bc	27.2 b	14.3 e
28	168	23.5 b	3.15 bc	27.3 b	11.9 bcd
112	168	21.0 b	2.94 b	26.3 b	12.4 cd
560	168	24.6 b	3.49 c	27.7 b	13.2 de
Average		<u>22.8 y</u>	<u>3.24 y</u>	<u>27.1 y</u>	<u>12.9 y</u>

^{1/}Values in the same vertical column followed by the same letter are not statistically different ($P < 0.05$).

Thus, Mn levels used in this experiment neither stimulated nor reduced growth of Douglas-fir seedlings.

Soil pH.--pH values ranged from a high of 6.1 to a low of 5.1 (table 2). Values varied little by date of measurement, and both Mn and N tended to decrease pH slightly. Appreciable pH changes in surface soil due to urea or manganous sulfate were not detected since soil samplings for pH measurements were to the full depth of the pots.

Available Mn in soil.--At harvest, there were still 7 to 26 p/m Mn available in the soil (table 3). Available Mn increased with additions of manganous sulfate. Applications of urea N, however, reduced available Mn; this effect is presumably associated with increased growth and uptake of Mn by nitrogen-fertilized seedlings.

Table 2--Effect of manganese and nitrogen treatments
on soil pH, by date of sampling

Treatment		pH		
Mn	N	5-4-77	7-19-77	1-19-78
<u>Kilograms per hectare</u>				
0	0	6.1	5.7	5.9
28	0	6.1	5.7	6.0
112	0	5.7	5.4	5.9
560	0	5.3	5.2	5.8
Average		<u>5.8</u>	<u>5.5</u>	<u>5.9</u>
0	168	6.0	5.4	5.8
28	168	5.8	5.5	5.8
112	168	5.7	5.4	5.7
560	168	5.4	5.1	5.7
Average		<u>5.7</u>	<u>5.4</u>	<u>5.8</u>

Table 3--Effect of manganese and nitrogen treatments
on available Mn in soil^{1/}

Treatment		Available Mn in soil
Mn	N	
<u>Kilograms per hectare</u>		<u>Parts per million</u>
0	0	7 a
28	0	11 a
112	0	12 a
560	0	26 b
Average		<u>14 x</u>
0	168	7 a
28	168	8 a
112	168	10 a
560	168	23 b
Average		<u>12 y</u>

^{1/}Values in the same vertical column followed by the same letter are not statistically different (P<0.05).

Chemical characteristics of foliage.--Applications of Mn, with and without additions of N, did not significantly affect levels of N, Ca, Fe, or Zn (table 4). Concentrations of Mn in foliage, on the other hand, were affected. Thus, Mn levels increased from 207 to 1,203 p/m with an increase of added Mn in absence of N and from 193 to 968 p/m when N was applied. Additions of N did not significantly affect foliar concentrations of any of the nutrients except Mn; average Mn levels were significantly lower with N than without. Effects of N additions were probably due to more dilution by growth with urea than without.

Table 4--Chemical characteristics of Douglas-fir seedlings treated with manganese and nitrogen^{1/}

Treatment		N	Ca	Mn	Fe	Zn
Mn	N					
Kilograms per hectare		- - Percent - -	- - - Parts per million - - -			
0	0	1.02 a	0.24 a	207 ab	156 ab	50 a
28	0	1.11 ab	.26 a	244 b	177 b	50 a
112	0	1.03 a	.25 a	338 c	117 ab	47 a
560	0	1.19 ab	.26 a	1,020 e	155 ab	56 a
Average		<u>1.09 x</u>	<u>.25 x</u>	<u>453 x</u>	<u>151 x</u>	<u>51 x</u>
0	168	1.34 b	.26 a	193 a	126 ab	44 a
28	168	1.28 ab	.27 a	188 a	141 ab	48 a
112	168	1.06 ab	.25 a	209 ab	143 ab	43 a
560	168	1.14 ab	.25 a	968 d	105 a	46 a
Average		<u>1.20 x</u>	<u>.26 x</u>	<u>390 y</u>	<u>129 x</u>	<u>45 x</u>

^{1/}Values in the same vertical column followed by the same letter are not statistically different (P<0.05).

Experiment 2

This experiment involved additions of very high levels of Mn and was initiated when highest Mn treatments in the first experiment failed to induce any symptoms of Mn toxicity.

Methods

Seedlings, pots, and soil used in this experiment were similar to those of experiment 1. We applied manganous sulfate in amounts equivalent to 0, 1 207, 3 018, 6 036, and 12 070 kg Mn/ha to each of five pots. The pots were watered and kept in the lathhouse as in experiment 1. We added excessive amounts of Mn to determine tolerance of Douglas-fir to Mn. Current year's needles were harvested 6 months after Mn was applied and were processed and analyzed for Mn and Fe as in experiment 1.

Results

Foliar levels of Mn increased with the increase of Mn added, and the highest level found was nearly 4,000 p/m (table 5). Concentrations of iron in the foliage, however, varied little among the treatments. Seedling appearance was the same in all pots. Seedlings did not show any visible symptoms of injury, indicating that Douglas-fir is quite tolerant to high levels of Mn in the soil.

Table 5--Effect of excessive supply of manganese in the soil on contents of Mn and Fe in Douglas-fir foliage

Mn additions			
MnSo ₄ ·H ₂ O, grams per pot	Kilograms Mn per hectare	Mn	Fe
		<u>Parts per million</u>	
0	0	174	37
4.6	1 207	745	31
11.5	3 018	1,590	32
23.0	6 036	1,680	25
46.1	12 070	3,970	32

Experiment 3

Because toxicity was not induced even when Mn was applied at rates equivalent to 12 000 kg/ha, we decided to determine the extent of Mn sorption in the experimental soil and other forest soils.

Methods

We used the following soils which represent the range of inherent productivity found in the Douglas-fir region: Tumwater sandy loam, Boistfort clay loam, Alderwood gravelly sandy loam, and Kinney Creek loam. Available native Mn in these soils varied widely, ranging from 17 p/m in Tumwater sandy loam to 136 p/m in Boistfort clay loam. We added manganous sulfate in amounts equivalent to 250 or 500 p/m Mn to 50-g portions of each of the four soils. The manganous sulfate was thoroughly mixed into the soil, and water was added to bring soil moisture to field capacity. The treated soils were covered and left to equilibrate at room temperature. After 4 days, Mn was extracted from the soils with DTPA, and Mn in the filtered extracts was determined by atomic absorption as in experiment 1. Native Mn in the soils was also determined and subtracted from total extracted Mn before percent Mn sorbed by the different soils was calculated.

Results

Sorption of Mn varied among and within the different soils, and percent sorption ranged from 30.8 to 64.0 (table 6). Percent sorption varied little between the 250 and 500 p/m treatments in the Tumwater sandy loam and Alderwood gravelly sandy loam but differed widely between the two treatments in the Boistfort clay loam and the Kinney Creek loam. Reasons for the latter differences are unexplainable but not unreasonable, based on other studies (e.g., Shuman et al. 1978). Over both treatments, Tumwater sandy loam had the lowest sorbed Mn and Boistfort clay loam the highest. Sorption appears to be strongly related to soil texture; increased sorption occurs in soils having a higher content of clay.

Table 6--Sorption of manganese by different soils

Soil	Mn added	Mn extracted ^{1/}	Mn sorbed
		<u>Parts per million</u>	<u>Percent</u>
Tumwater sandy loam	250	173	30.8
Tumwater sandy loam	500	342	31.6
Boistfort clay loam	250	120	52.0
Boistfort clay loam	500	190	64.0
Alderwood gravelly sandy loam	250	162	35.2
Alderwood gravelly sandy loam	500	302	39.6
Kinney Creek loam	250	157	37.2
Kinney Creek loam	500	221	55.8

^{1/}Excludes extracted native manganese.

Experiment 4

Tests in soil did not demonstrate Mn toxicity in Douglas-fir. Since considerable Mn sorption may occur in some forest soils, we investigated the effects of varying levels of Mn in hydroponic culture.

Methods

We used a series of 0.1-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) containing different concentrations of Mn to test tolerance of Douglas-fir to Mn and to determine critical levels of Mn and toxicity symptoms. We used manganous sulfate to obtain Mn concentrations of 0.05, 20, 40, 100, 200, 400, 1,000, and 2,000 p/m. Treatment solutions were contained in quart mason jars covered with aluminum foil. Two 1-year-old Douglas-fir seedlings were suspended in each jar. The seedlings were in a state of active growth, and the jars were placed in a plant growth chamber. Temperatures in the chamber were 26°C during the day and 16°C at night; a 14-hour photoperiod was maintained. Culture solutions were continuously aerated and were changed after 15 days; seedlings were harvested after 1 month. Roots of treated plants were thoroughly washed in distilled water, and plants were separated into roots and shoots at the root collar. Composite samples of shoots and roots were obtained by combining shoots and then roots of each two seedlings of each treatment, and the different composite parts were ground to 40 mesh in a Wiley mill. Levels of Mn in the ground roots and shoots were determined by atomic absorption as in experiment 1.

Results

Roots and shoots of seedlings of all treatments appeared normal until 4 days after the experiment was started, when needles of seedlings receiving the 400, 1,000, and 2,000 p/m Mn showed some symptoms of toxicity. These symptoms consisted of wilting of the needles at the tips of the main stem and lateral branches, followed by death and desiccation of the wilted needles. With time, toxicity progressed from the tips of the stems and branches toward the bases; by the end of the experiment, large portions of the shoots of the affected seedlings were dead. Roots were also affected by excess Mn; they appeared blackened and lacked elongation compared with those of normal seedlings. Toxic effects on both shoots and roots increased as the concentration of Mn in the nutrient solution was increased from 400 to 2,000 p/m.

Roots and shoots of all seedlings contained Mn (table 7). Levels in the shoots increased as concentrations of Mn in the nutrient solution were increased. Manganese in the roots also increased but reached its highest level at 200 p/m Mn in the nutrient solution. In seedlings which showed toxicity symptoms, Mn ranged from 20,700 to 40,000 p/m in the shoots and from 1,540 to 2,320 p/m in the roots. Seedlings that did not show toxicity symptoms tolerated a nutrient solution containing 200 p/m Mn; concentrations were 8,100 p/m in shoots and 2,410 p/m in roots.

Table 7--Effect of different concentrations of manganese in nutrient solution on levels of Mn in shoots and roots of Douglas-fir seedlings

Mn concentration in nutrient solution	Mn in shoots	Mn in roots
<u>Parts per million</u>		
0	151	192
20	1,760	996
40	2,900	1,320
100	6,650	1,870
200	8,100	2,410
400	20,700	2,320
1,000	32,100	1,540
2,000	40,000	2,320

DISCUSSION AND CONCLUSIONS

Our studies indicate that Douglas-fir has high tolerance for Mn in soil, with and without additions of urea, and in nutrient solution culture. Applications of manganous sulfate to the soil in amounts equivalent to more than 12 000 kg Mn/ha resulted in concentrations of about 4,000 p/m Mn in foliage; but seedling growth was not reduced, nor were there visual symptoms of injury. Similarly, toxicity symptoms were not observed in solution culture at concentrations of up to 200 p/m Mn; under these conditions, the seedlings tolerated about 8,000 p/m Mn in their shoots and 2,400 p/m in their roots.

Results of the sorption experiment show that forest soils have capacity to fix or tie up Mn. Although sorption data obtained were not as high as those determined in agricultural soils (Shuman et al. 1978), significant amounts of Mn were sorbed by the soils used. Such sorption may limit accumulation of excess available Mn in soil and reduce the probability of Mn toxicity. This, together with the high tolerance of Douglas-fir for Mn mentioned above, suggests that it is unlikely that Mn toxicity can be important in management of Douglas-fir forests.

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KEYWORDS: Soil manganese, nitrogen fertilizer response, seedling growth, Douglas-fir, Pseudotsuga menziesii.

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